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The vascular endothelin system is not overactive in normotensive hemodialysis patients

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The vascular endothelin system is not overactive in normotensive hemodialysis patients.

Background. The hemodynamic significance of elevated endothelin-1 (ET) plasma levels in hemodialysis (HD) patients is unknown. Therefore, we studied the role of ET in the regulation of vascular tone in normotensive HD patients and matched healthy controls (C).

Methods. The forearm blood flow (FBF) responses to adenosine, norepinephrine, the ET-A receptor antagonist BQ-123 (40 nmol/min), the ET-B receptor antagonist BQ-788 (1 and 50 nmol/min), and ET (5 pmol/min) were measured. Results are percent of baseline change ± SEM (baseline = 100%).

Results. Responses to adenosine and norepinephrine were both unchanged in HD. In HD, BQ-123 increased FBF less than in C (133 ± 9 vs. 178 ± 27%; $P = 0.02$). BQ-788 failed to change FBF in C but decreased FBF to 83 ± 4% in HD. Compared to BQ-123 alone, BQ-123 plus BQ-788 (50 nmol/min) caused an additional increase of FBF (234 ± 32%, $P < 0.001$) in C, but not in HD (139 ± 14%). This additional increase was absent when BQ-788 was co-infused at 1 nmol/min. ET reduced FBF comparably in both groups.

Conclusions. Resistance vessels of HD patients have unremarkable contractile properties, as shown by responses to adenosine and norepinephrine. In HD, the basal vascular ET-mediated tone is reduced. The main action of the ET-B receptor in C is vasoconstrictive, which also is blunted in HD. The intact response to exogenous ET indicates the normal function of ET receptors in HD. Our results could be explained by a reduced generation or reduced metabolic clearance rate of ET in normotensive HD patients. Controversy remains concerning the role of the ET-B receptor when comparing the present data with previously published literature.

Endothelin-1 (ET) is an endogenous peptide with potent properties of a vasoconstrictor. It is primarily secreted by endothelial cells [1]. ET has been shown to contribute to basal vascular tone and blood pressure in healthy humans [2]. The effects of ET are mediated by at least two different receptors [3]: An ET-A receptor on vascular smooth muscle cells causes vasoconstriction [4], while in contrast, an ET-B receptor on endothelial cells is related to the release of nitric oxide, prostaglandin I_2, and functional vasodilation [5]. ET-B receptors also are expressed on vascular smooth muscle cells (VSMC) where they cause vasoconstriction in specific situations [6]. In addition to vasomotion, ET stimulates proliferation and hypertrophy of VSMC; these effects are mediated by ET-A and ET-B receptors [5].

In chronic renal failure measurable plasma concentrations of ET and big-endothelin-1 are known to be elevated [7–10], and this led to the theory that ET antagonists hold great promise for the treatment of renal diseases, including renal failure [11]. However, the significance of elevated ET in chronic renal failure is not clear. For example, Demuth et al determined ET levels and studied changes in chronic hemodialysis patients [12]. The authors reported a significant correlation between plasma ET and cardiovascular remodeling in their patients. Similar observations were made by Nabokov et al, who tested an animal model of arteriosclerosis in chronic renal insufficiency [13]. On the other hand, Ottosson-Seeberger et al attempted to correlate measurements of ET concentrations in plasma in HD patients with mean arterial blood pressure, but no correlation was discernible [9]. Hand et al performed functional studies of ET in patients with chronic pre-dialysis renal insufficiency who were also hypertensive. The authors found evidence of a reduced—rather than an increased—ET-mediated vascular tone [14].

The present studies examined these controversies as well as the functional role of ET specifically in chronic normotensive HD patients, which has not been described previously. Normotensive patients receiving chronic hemodialysis were selected for study because hypertension is known to alter the ET system by increasing the ET-

Key words: chronic renal failure, hemodialysis, endothelin receptors, forearm blood flow, resistance vessels, uremia.

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mediated arterial tone [15]. Forearm blood flow (FBF) in arterial resistance vessels was measured by strain gauge plethysmography, as previously reported by our group [16]. Our protocol first sought to establish the response of arterial resistance vessels to ET-independent stimuli of vasomotion, such as adenosine and norepinephrine. The concentrations of ET were measured in peripheral venous plasma. Patients were infused with the ET-A receptor antagonist BQ-123 and the ET-B receptor antagonist BQ-788 into the brachial artery. We also infused exogenous ET.

METHODS

The study protocol was approved by the ethics committee of our institution. All participants gave written informed consent.

Study subjects

All participants in the various protocols of this study were taken from an initial population of 15 HD and 15 control subjects. Renal failure was due to chronic glomerulonephritis ($N = 6$), chronic pyelonephritis ($N = 3$), reflux nephropathy ($N = 2$), interstitial nephritis ($N = 2$), and autosomal dominant polycystic kidney disease ($N = 2$). Nine HD and eleven control subjects underwent all parts of the ET-blocker protocols, while six HD patients and eight control subjects underwent all protocols. Hemodialysis (HD) patients were recruited from local dialysis centers. Patients had been receiving regular (3×, 4 to 5 h/week) hemodialysis treatment for at least one year. Patients were included only when their dry weight had been unchanged for the previous four weeks. Dry weight was assessed by: (*1*) ultrasound of the inferior vena cava, and (*2*) an absence of edema, dyspnea, intradialytic cramps and drop of blood pressure. HD patients were normotensive as documented by normal blood pressure measurements taken by the dialysis staff before the beginning of the individual dialysis sessions over the preceding three months (with systolic blood pressure <130 mm Hg and diastolic blood pressure <85 mm Hg). None of the HD patients was taking antihypertensive medication or non-steroidal anti-inflammatory drugs (NSAID). HD patients included in the study did not have any known evidence of arteriosclerosis. In this respect we documented an absence of the following: angina pectoris; signs of coronary artery disease and chronic heart failure by electrocardiogram (EKG) or echocardiography; history of cerebrovascular accident or transient ischemic attack; and claudication of the legs. HD patients were excluded from the study when they had diabetes mellitus, hypercholesterolemia, liver cirrhosis, or a history of smoking. Recombinant human erythropoietin (rhEPO) treatment is common in HD patients, and our study cohort had an unchanged rhEPO treatment for at least 12 weeks prior to the study's start (Table 1).

Healthy volunteers matched for sex, age and body weight were studied as controls. None was taking medication. All were normotensive (blood pressure criteria the same as for HD patients). General characteristics of the participants in the study are in Table 1.

Protocol of investigation

All studies were performed in a quiet temperature-controlled room (temperature 22 to 25°C) between 9 a.m. and 4 p.m. with the subjects in a supine position. All subjects had been asked to refrain from large meals and from beverages that contained alcohol or caffeine during the six hour interval before the study. HD patients were studied during the interdialytic day (12 to 24 hours after the preceding dialysis). Care was taken that their dry weight did not exceed 1.5 kg. A 27 SWG steel cannula (Cooper's Needle Works, Birmingham, UK) was inserted into the brachial artery. The non-dominant arm was used in the control subjects and the fistula-free arm in HD patients. Forearm blood flow (FBF) was measured by strain gauge plethysmography (Gutmann Medizinelektronik, Eurasburg, Germany) as published previously [16]. In control subjects FBF was measured in both arms, while in HD patients the measurement of FBF was restricted to the cannulated arm because of the fistula in the opposite arm. Individual measurements of FBF were made over three minutes for each determination of FBF. For this purpose upper arm cuffs were intermittently inflated to 40 mm Hg for 10 seconds every 15 seconds to temporarily prevent venous outflow from the forearm and thus obtain plethysmographic recordings. During periods of measurement, the blood flow of the hand(s) was excluded by a wrist cuff inflated to a suprasystolic pressure. After cannulation of the brachial artery, saline was injected first during 30 minutes of equilibration. Thereafter at least two baseline determinations of FBF were made. Subsequently, the study drugs were infused over 60 minutes according to protocols 3 to 6, described in the next sections. FBF was measured every 10 minutes; arterial blood pressure was measured at baseline and at the end of each protocol. All agents used were dissolved in 0.9% saline. During the entire study the rate of infusion was constant at 1 mL/min.

Protocols 3 to 6, which involved infusions of ET antagonists or ET alone were performed on separate days and at least one week apart.

Protocol 1: Vascular response to adenosine

After measurement of baseline FBF 10 HD patients and 10 healthy control subjects received an intra-arterial infusion of incremental doses of adenosine (75, 150, 300 μg/min). Increasing doses were infused subsequently with a five-minute period of infusion for each dose. These

doses previously have been shown to cause effective local vasodilation in the human forearm [17].

Protocol 2: Vascular response to norepinephrine

Six HD patients and eight healthy control subjects subsequently received three increasing doses of norepinephrine (60, 120, 240 pmol/min). Each dose was infused over 10 minutes before FBF was measured.

Protocol 3: Vascular response to the ET-A receptor blockade

After the determination of baseline FBF values, 9 HD patients and 14 control subjects received an intra-arterial infusion of the selective ET-A receptor antagonist BQ-123 (Clinalfa, Läufelfingen, Switzerland), at a dose of 40 nmol/min. The infusion was given over 60 minutes. This dose has been shown in previous studies in the human forearm to fully block the ET-A receptor-mediated effects of ET locally, that is in the forearm, without systemic effect [18].

Protocol 4: Vascular response to the ET-B receptor blockade

Ten HD patients and 12 control subjects received an intra-arterial infusion of the selective ET-B receptor antagonist BQ-788 (Clinalfa) for 60 minutes. The dose was 50 nmol/min, which has been shown to effectively block the ET-B receptor locally in the human forearm [15]. The expected concentration of BQ-788 at its receptor was tenfold higher than the pA_2 for BQ-788. The pA_2 indicated a twofold shift of the dose-response curve of endothelin-1 to the right at this receptor [19].

Protocol 5: Vascular response to the combined ET-A/ET-B receptor blockade.

Ten HD patients and 11 control subjects received simultaneous intra-arterial infusion of BQ-123 and BQ-788 (dosages as reported in protocols 3 and 4). As a 50-fold lower dose of BQ-788 had been used to block the ET-B receptor-mediated tone in a prior study by Verhaar and coworkers [18], we repeated the combined infusion of BQ-123 and BQ-788 in nine healthy control subjects, using the lower dose (1 nmol/min BQ-788).

Protocol 6: Vascular response to exogenous ET-1

Nine HD patients and eight control subjects received intra-arterial ET (Clinalfa) at a dose of 5 pmol/min for 60 minutes. This dose has been shown previously to cause vasoconstriction in healthy subjects and in patients with renal insufficiency [14].

Measurement of plasma levels of ET-1

Peripheral venous blood was drawn into chilled ethylenediaminetetraacetic acid (EDTA) tubes after patients had been supine for 30 minutes. The tubes were placed on ice immediately. Plasma was separated within three to five minutes by centrifugation at 3°C and stored at −80°C. Plasma levels of immunoreactive ET were measured using an ELISA (Biomedica, Vienna, Austria). Recovery of ET-1 was 95%. Reported intra-assay and interassay coefficients of variation were 4.5% and 6.9%, respectively; the sensitivity of the assay was 0.05 fmol/mL. Reported cross reactivities of this ET assay were: ET-1 100%, ET-2 100%, ET-3 <5%, and big-ET <1%. As the assay is highly specific for ET-1 and ET-2 and the latter is not secreted into the blood in humans, the assay as used in our study may be considered an assay of plasma ET-1 concentration [20].

Calculations and statistical analyses

All data are reported as mean ± SEM. For each determination of FBF the mean of the last 10 individual FBF measurements for each test period was calculated. Observations are reported as percent of baseline FBF (FBF observed × 100/baseline FBF). For statistical analyses of dose-response relationships, a two-way analysis of variance (ANOVA) for repeated measurements was used according to the SPSS™ software program (SPSS Inc., Chicago, IL, USA). A value of $P < 0.05$ was considered significant. Bonferroni correction for multiple testing was performed where appropriate. All other parameters were analyzed by the Student paired or unpaired *t* test as appropriate.

RESULTS

As shown in Table 1, HD patients and controls were comparable in terms of age, sex, gender, height, weight and total cholesterol. However, as indicated in each protocol, the hematocrit values were significantly lower in HD patients than in control subjects. Mean arterial blood pressure and heart rate were not different between these groups at baseline, nor did they change significantly after the different periods of infusion for control and HD subjects. There were no side effects or complications during the study. In controls FBF in the non-infused arm remained constant throughout each protocol (Table 2), indicating that the locally infused agents did not cause systemic effects. Baseline FBF values were similar in both groups (Table 2). In each protocol FBF in the infused arm was comparable between both groups. However, in HD patients the baseline FBF in the adenosine protocol was significantly lower than in the protocols where ET antagonists were administered (Table 2).

Immunoreactive ET plasma levels were significantly higher in HD patients than in controls (3.9 ± 0.3 pg/mL, $N = 24$ vs. 2.7 ± 0.2 pg/mL, $N = 23$; $P < 0.05$). No correlations were found between the ET plasma levels and: (*a*) the dose of rhEPO; (*b*) responses to ET, BQ-

Table 1. Subject characteristics

Characteristic	BQ-123		BQ-788		BQ-123/BQ-788		Endothelin-1		Adenosine		Norepinephrine	
	HD	Control	HD	Control	HD	Control	HD	Control	HD	Control	HD	Control
Number	9	14	10	12	10	11	9	8	8	8	6	8
Sex *M/F*	7/2	11/3	8/2	11/1	8/2	10/1	7/2	6/2	6/2	6/2	6/0	7/1
Age *years*	40.8 ± 4.1	41.2 ± 4.5	40.8 ± 4.8	40.6 ± 4.2	40.8 ± 3.9	40.9 ± 4.2	40.6 ± 4.1	41.8 ± 5	40.6 ± 4.1	40.9 ± 3.6	41.2 ± 2.9	40.9 ± 2.2
Height *cm*	172 ± 3.8	171 ± 3.9	169 ± 4.2	170 ± 3.9	172 ± 4.2	173 ± 3.9	170 ± 4	171 ± 4.2	172 ± 3.6	170 ± 3.9	171 ± 2.3	172 ± 2.1
Weight *kg*	66.3 ± 3.9	65.2 ± 4.4	66.2 ± 4.1	66.7 ± 4.3	65.9 ± 3.8	66.1 ± 4	66.2 ± 3.9	66.4 ± 4.1	67.1 ± 3.6	67.3 ± 3.1	66.4 ± 2.6	67.3 ± 2.2
Mean arterial pressure *mm Hg*	90 ± 3	91 ± 3.5	90 ± 3.5	89 ± 4.2	89 ± 3.9	90 ± 4.1	91 ± 3.7	90 ± 3.8	89 ± 3.2	90 ± 3.7	91 ± 3.2	90 ± 2.7
Total cholesterol *mmol/L*	5.1 ± 0.3	5.2 ± 0.4	5.0 ± 0.4	5.1 ± 0.6	5.0 ± 0.3	5.1 ± 0.4	5.2 ± 0.3	5.1 ± 0.3	5.0 ± 0.4	5.1 ± 0.5	5.1 ± 0.3	5.2 ± 0.3
Hematocrit	0.36 ± 0.02[a]	0.42 ± 0.01	0.35 ± 0.01[a]	0.42 ± 0.02	0.35 ± 0.03[a]	0.42 ± 0.03	0.35 ± 0.02[a]	0.43 ± 0.02	0.36 ± 0.02[a]	0.44 ± 0.03	0.37 ± 0.04[a]	0.44 ± 0.02
Erythropoietin *dose: I.U. per week*	3128 ± 148		3094 ± 94		3012 ± 124		3002 ± 98		3102 ± 112		3002 ± 102	
Erythropoietin +/−	7/2		7/3		8/2		7/2		7/1		5/1	
Time on HD *months*	81.1 ± 4.1		79.8 ± 3.8		82.4 ± 4.2		81.2 ± 3.9		80.3 ± 4.1		80.6 ± 3.9	

[a] $P < 0.05$ compared to Control

123, BQ-788, and the combined infusion of BQ-123 and BQ-788; or (*c*) pulse pressure.

Vascular response to adenosine

Infusion of the vasodilator adenosine caused a dose-dependent increase in FBF (HD, 339 ± 50%, 530 ± 81%, 656 ± 110%; control, 267 ± 40%, 426 ± 61%, 584 ± 110% for doses 1 to 3, respectively). These responses were comparable for HD patients and control subjects.

Vascular response to norepinephrine

Norepinephrine caused a similar dose-dependent reduction of FBF in both groups (HD patients, 85 ± 3%, 71 ± 5%, 52 ± 5%; control, 83 ± 4%, 67 ± 4% and 56 ± 5% for doses 1 to 3, respectively).

Vascular responses to ET-A receptor blockade

Infusion of BQ-123 caused a progressive increase of FBF in both groups that was maximal at 60 minutes. It increased FBF in control subjects by 178 ± 27% and in HD patients by 133 ± 9% ($P = 0.02$; Fig. 1). The increase in FBF was significantly attenuated in HD patients compared to control subjects.

Vascular response to ET-B receptor blockade

There was no significant difference in the response to BQ-788 between HD and control subjects (Fig. 2). However, when the HD patients were analyzed on their own vasoconstriction to 83.13 ± 4.31 was observed ($P < 0.05$).

Vascular response to combined ET-A/ET-B receptor blockade

Simultaneous infusion of BQ-123 and BQ-788 (50 nmol/min) caused a progressive increase in FBF in both groups (Fig. 3A). BQ-123 plus BQ-788 increased FBF significantly more in control subjects (234 ± 32%) than in HD patients (139 ± 14%; $P < 0.001$; Fig. 3A). In control subjects, but not in HD, the increase in FBF to the combined infusion of BQ-123 and BQ-788 was significantly greater than that to BQ-123 alone ($P < 0.01$; Fig. 3C).

When BQ-788 was co-infused at the lower dose (1 nmol/min) in nine healthy control subjects (all of whom had received BQ-123 alone and BQ-123 and BQ-788 at the higher dose; Fig. 3B), combined infusion of BQ-123 and BQ-788 increased FBF to the same extent as BQ-123 alone and significantly less than the combined infusion with BQ-788 at the higher dose (Fig. 3B).

Vascular response to exogenous ET-1

Infusion of ET caused a progressive vasoconstriction to approximately 50% in both HD and C. In HD the dose response curve was shifted to the left ($P < 0.05$)

Table 2. Response of forearm blood flow to different infusions in control and hemodialysis patients

Variable	Control subjects		HD patients
	Control arm FBF *mL/100 mL × min*	Infused arm FBF *mL/100 mL × min*	Infused arm FBF *mL/100 mL × min*
Baseline	2.4 ± 0.3	2.6 ± 0.3	2.6 ± 0.4
BQ-123	2.6 ± 0.4	4.4 ± 0.7	3.4 ± 0.5
Baseline	2.3 ± 0.4	2.5 ± 0.4	2.8 ± 0.2
BQ-788	2.6 ± 0.4	2.6 ± 0.4	2.3 ± 0.1
Baseline	2.4 ± 0.4	2.1 ± 0.3	2.2 ± 0.5
BQ-123/BQ-788	2.4 ± 0.3	4.6 ± 0.5	3.1 ± 0.4
Baseline	2.3 ± 0.3	2.2 ± 0.3	
BQ-123/BQ-788$_{low}$	3.2 ± 0.8	5.1 ± 1.1	
Baseline	2.6 ± 0.2	2.9 ± 0.5	3.3 ± 0.5
Endothelin-1	2.8 ± 0.3	1.5 ± 0.2	1.8 ± 0.4
Baseline norepinephrine	2.0 ± 0.3	2.2 ± 0.3	2.4 ± 0.4
60 pmol/min	1.9 ± 0.3	1.8 ± 0.3	2.1 ± 0.4
120 pmol/min	1.9 ± 0.2	1.5 ± 0.2	1.8 ± 0.3
240 pmol/min	2.0 ± 0.3	1.3 ± 0.2	1.3 ± 0.3
Baseline adenosine	1.8 ± 0.3	2.0 ± 0.4	1.6 ± 0.3
75 μg/min	1.8 ± 0.4	4.8 ± 1.0	5.3 ± 1.0
150 μg/min	1.7 ± 0.3	7.2 ± 1.2	7.6 ± 1.0
300 μg/min	1.8 ± 0.3	10.3 ± 1.9	9.3 ± 1.2

Forearm blood flow (FBF) was in response to intra-arterial infusions of the ET-A receptor antagonist BQ-123 (40 nmol/min), the ET-B receptor antagonist BQ-788 (50 nmol/min), and the combined ET-A/ET-B receptor blockade by BQ-788 (50 nmol/min) plus BQ-123 (40 nmol/min), endothelin-1, norepinephrine and adenosine. Note that in control subjects the combined infusion of BQ-123 and BQ-788 was repeated with BQ-788 included at 1 nmol/min. This is indicated by BQ-123/BQ-788$_{low}$. Data are given at baseline and at 60 minutes (for adenosine and noreepinephrine at the end of infusion of each dose) for each protocol. For control subjects the forearm blood flow also is reported for the contralateral arm, indicating potential systemic effects.

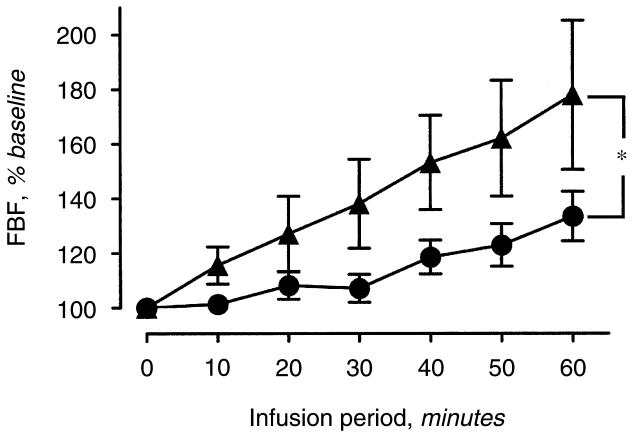

Fig. 1. Changes in forearm blood flow in response to intra-arterial infusion of BQ-123 (40 nmol/min) in 9 hemodialysis (HD) patients (●) and 14 healthy volunteers (controls; ▲). Baseline = 100%; values are mean ± SEM. The *P* value refers to the comparison between the two groups by two-way ANOVA for repeated measurements.

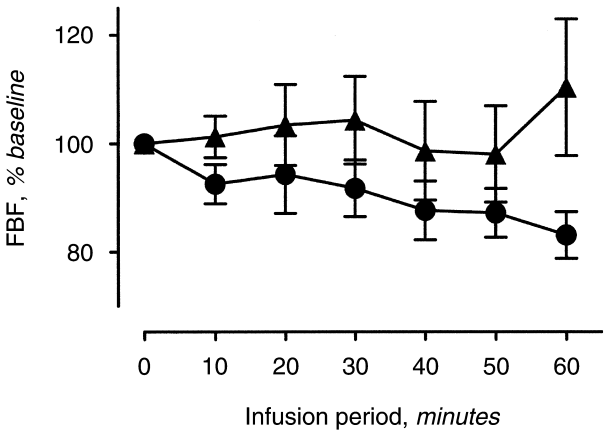

Fig. 2. Changes in forearm blood flow in response to intra-arterial infusion of BQ-788 (50 nmol/min) in 10 HD patients (●) and 12 healthy volunteers (controls; ▲). Baseline = 100%; values are mean ± SEM.

compared to that of C. This indicates greater sensitivity to ET in HD patients. Maximal observed effects at 60 minutes, however, were comparable in HD and C (HD patients, 52 ± 6%; control subjects, 55 ± 5%; Fig. 4).

DISCUSSION

This study was designed to describe the functional state of the ET system in normotensive patients on chronic HD. Carefully matched control subjects were studied as well. ET has been suggested to play a pivotal role in vascular remodeling and in the development of arteriosclerosis in these patients. Excessive cardiovascular mortality is well known in HD patients [21]. We confirmed that plasma ET levels are elevated in normotensive HD patients, as has been reported previously [7–10]. However, the functional role of this ET in vasomotion in HD patients is not clear. Therefore, we studied the local hemodynamic effects of these elevated plasma ET levels in normotensive HD patients. Normotensive patients were examined in an effort to exclude possible confounding factors such as hypertension where increased ET-mediated vascular tone has been shown [15].

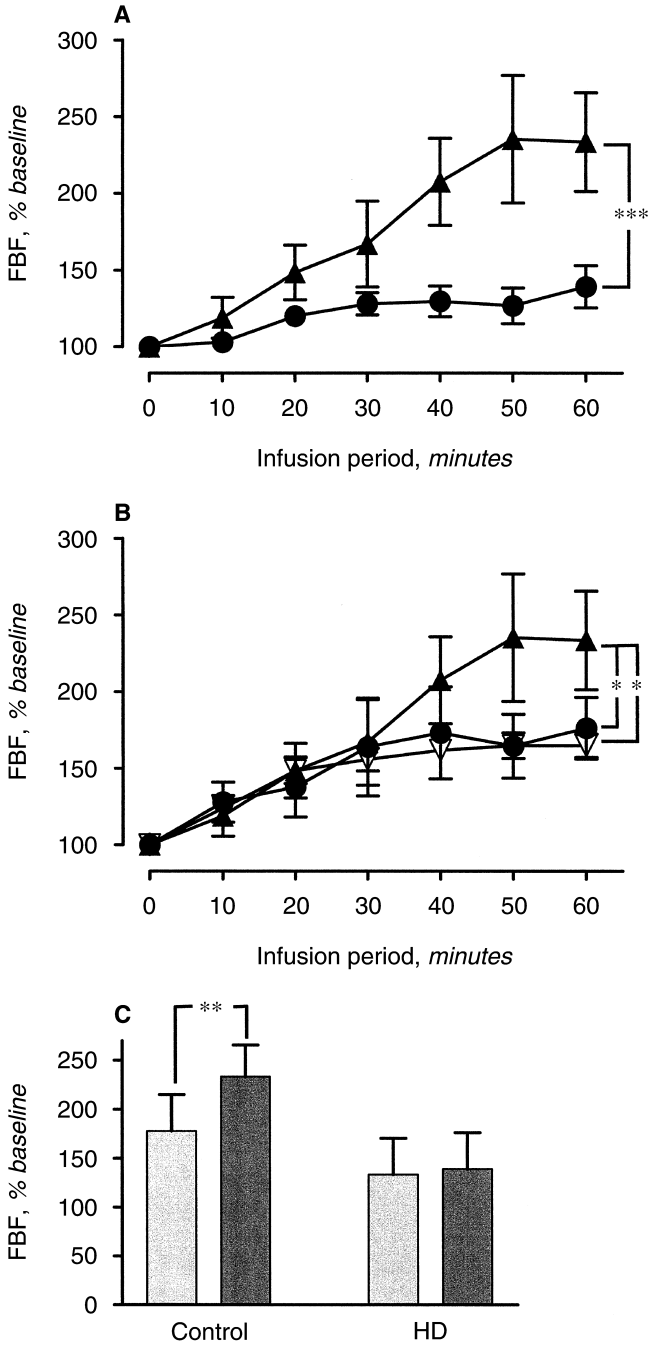

Fig. 3. (*A*) Changes in forearm blood flow in response to combined intra-arterial infusion of BQ-123 (40 nmol/min) and BQ-788 (50 nmol/min) in 10 hemodialysis patients (HD; ●) and 11 healthy volunteers (CTL; ▲). Baseline = 100%; values are mean ± SEM. ***$P < 0.001$ by two-way ANOVA for repeated measurements for comparison between HD and CTL. (*B*) Changes in forearm blood flow in response to combined intra-arterial infusion of BQ-123 (40 nmol/min) and BQ-788 (1 nmol/min) in healthy volunteers (L; ▽) compared to combined infusion of BQ-123 (40 nmol/min) and BQ-788 (50 nmol/min; H; ▲) and compared to infusion of BQ-123 (40 nmol/min; 123; ●) alone. *$P < 0.05$ for H vs. L and for H vs. 123, by two-way ANOVA for repeated measurements. (*C*) Maximal changes in forearm blood flow in response to combined intra-arterial infusion of BQ-123 (40 nmol/min) and BQ-788 (50 nmol/min) in 10 HD patients (HD) and 11 healthy volunteers (Control) and in response to BQ-123 (40 nmol/min) alone in 9 HD patients and in 14 healthy volunteers. Symbols are: (■) BQ-123; (▩) BQ-123 + BQ-788; **$P < 0.01$ by the Student unpaired *t* test.

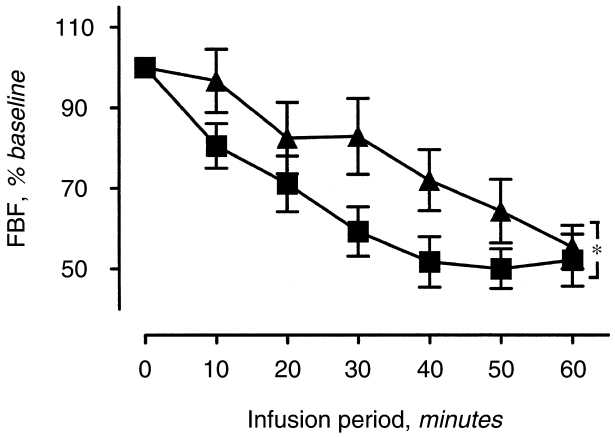

Fig. 4. Changes in forearm blood flow in response to intra-arterial infusion of endothelin-1 (5 pmol/min) in 9 HD patients (■) and 8 healthy volunteers (▲). Baseline = 100%; values are mean ± SEM. The *P* value refers to comparison between the two groups by two-way ANOVA for repeated measurements. The effect at 60 minutes was similar in both groups; however, the dose-response curve of HD was significantly shifted to the left.

For similar reasons, those patients with arteriosclerosis, diabetes mellitus and hypercholesterolemia and smokers also were excluded.

First, ET-independent vascular stimuli were tested to assess any potentially present general changes of vascular properties in HD patients. Our results obtained by the infusion of the vasodilator adenosine and the vasoconstrictor norepinephrine (NE) showed that the general vasomotive properties were intact and unchanged in HD. The finding of unchanged NE-mediated vasoconstrictor tone compared to healthy control subjects was in line with data obtained by Hand and coworkers [22]. Of note, this applied to patients treated with rhEPO whereas patients without rhEPO showed reduced norepinephrine-induced constriction. However, our study was not able to show a correlation between the vasoconstrictor response to NE and the dose of rhEPO administered, possibly due to a small number of patients without rhEPO treatment as well as the fact that our study was not designed to test this hypothesis. The ET was then infused, and the results showed that (*a*) at the dose chosen for our experiments, its dominant effect in HD was vasoconstrictive, and (*b*) there was no difference of effect at 60 minutes of infusion between HD and control. We therefore concluded that ET receptors were reactive and intact in HD. These data do not support any potential down-regulation or post-receptor desensitization of ET receptors in HD. Our finding that time-dependent vasoconstriction in HD patients was significantly shifted to the left, suggesting a higher sensitivity to exogenously applied ET, is in line with recent results obtained in isolated resistance arteries from uremic patients [23].

Infusions of BQ-123 alone or in combination with BQ-

788 caused substantial increases in FBF in HD patients, which showed the existence of a reasonable ET-mediated tone in the forearm resistance vessels of normotensive HD patients. Compared to control subjects, however, the increase of FBF in HD was significantly blunted in both protocols: infusion of BQ-123 alone and infusion of BQ-123 plus BQ-788. Together these data show a reduced endogenous ET-mediated tone in otherwise unremarkable arterial resistance vessels of normotensive HD patients.

Our observations may be explained by (*a*) a reduced generation of ET together with a reduced clearance of ET, and (*b*) inactivation or cleavage of bioactive ET or by other mechanisms. We did not perform additional experiments to further clarify the potential mechanisms for the reduced ET-mediated tone in normotensive HD. However, work done by other groups suggests that the effect of endogenous ET may be reduced in chronic renal failure. Hand, Haynes and Webb found a reduced vasodilation in response to ET-A receptor antagonism [14]; but unlike our current study their patients had chronic renal insufficiency not requiring dialysis treatment (mean serum creatinine 400 to 450 μmol/L).

The best possible explanation for our observations appears to be that normotensive HD patients have a reduced secretion of ET. This is supported by previous work in vitro that demonstrated a decrease of ET production by porcine endothelial cells upon exposure to serum from uremic patients [24]. Furthermore, Hand et al suggested that in predialysis chronic renal failure ET-generation is reduced, too [14]. Taken together, the data from the literature and our study argue in favor of a reduced ET generation in patients with renal failure.

In this view the elevated concentrations of ET-1 in the peripheral venous plasma of HD patients seem to be perplexing. However, Ottosson-Seeberger et al recently demonstrated a remarkable decrease of the metabolic clearance rate for ET in uremia [9]. They found that the plasma half-life of exogenous ET was increased in HD patients; they also proposed that the effective concentration of ET at its site of action, that is, the subendothelial space, and its measurable concentration in peripheral venous plasma were not correlated. This is in line with the absent correlation between ET plasma levels and the response to ET-A antagonism or combined ET-A and ET-B receptor antagonism found in our study. We therefore suggest that elevated plasma levels of ET in our HD patients are the result of decreased metabolic clearance.

In controls, our observations involving the combined application of BQ-123 plus BQ-788 (50 nmol/min) demonstrated a role of the ET-B receptor to cause vasoconstriction. This is at variance with data reported by Verhaar et al, who showed vasoconstriction for the ET-B receptor blockade indicating a vasodilatory effect of the ET-B receptor in healthy volunteers. Their experiments used selective ET-B receptor blockade and nonspecific ET-A and ET-B receptor blockade [18]; however, the dose of BQ-788 used in their study was 50-fold lower than the dose used in our current study. On the other hand, Cardillo et al showed an absence of vasoconstriction in response to ET-B receptor blockade in healthy volunteers [25]. As in our study, they used BQ-788 at a dose of 50 nmol/min, which was 50-fold higher than that used by Verhaar and coworkers. To clarify whether these differences were due to a dose effect, we repeated the combined infusion of BQ-123 with BQ-788 experiments at 1 nmol/min in healthy control subjects. At this dose an additional increase in FBF compared to BQ-123 alone was absent. However, we did not observe a blunted increase in FBF compared to the infusion of BQ-123 alone, as was shown by Verhaar. There are at least two conceivable possibilities to explain the discrepancies between the different doses of BQ-788: BQ-788 as high as 50 nmol/min could cause an additional ET-A receptor blockade that results in a further increase in flow. We did not perform studies to exclude non-specific blockade of ET-A receptors by this high dose of BQ-788. While Verhaar et al demonstrated that BQ-123 maximally blocked ET-A receptors at a dose of 10 nmol/min, our study used BQ-123 at a dose of 40 nmol/min. This indicates that ET-A receptors may have been maximally blocked by the dose of BQ-123 used in our study and therefore an additional non-specific blockade is unlikely. Moreover, the known specificity of BQ-788 for the ET-B receptor is very high and our dose was chosen to achieve a local concentration in the forearm at least tenfold higher than the pA_2 at the ET-B receptor [19]. Another explanation would be a dose-dependent effect of BQ-788 on ET-B receptors in different locations [19]. This could mean that, at the lower dose, BQ-788 blocks only endothelial ET-B receptors with subsequent vasoconstriction due to decreased ET clearance and reduced nitric oxide production. At higher doses BQ-788 also could block myocyte ET-B receptors, leading to net vasodilation and increase in local forearm blood flow. However, our experimental setting does not allow further elucidation of this topic. In another study, Strachan et al recently showed that ET-B receptor agonism by BQ-3020 or sarafotoxin s6c causes vasoconstriction in the forearm circulation in healthy volunteers [26]. Therefore, these data also point to a vasoconstrictor effect of ET-B receptor activation.

In control subjects BQ-788 (50 nmol/min) did not modify local forearm blood flow. This appears to be perplexing. However, as in this setting the vasoconstrictive ET-A receptor is unopposed, the vasodilator effect of ET-B receptor antagonism may be blunted by displacement of ET to the ET-A receptor. The net effect might be an unchanged forearm circulation. In HD pa-

tients ET-B receptor antagonism results in vasoconstriction. As the non-selective ET-A and ET-B receptor blockade showed a reduced vasoconstrictive ET-B receptor tone in HD patients compared to controls, selective ET-B receptor antagonism results in vasoconstriction due to activation of unopposed ET-A receptors.

Finally, although controversy remains concerning the effect of the ET-B receptor in the forearm circulation, this does not change our main finding that the activity of the ET system is reduced in uremia.

Hemodialysis patients commonly receive rhEPO as part of their treatment, and this applied to our patients, too. The influence of rhEPO on ET secretion is somewhat controversial. It has been shown in cell culture studies and in isolated vessels that rhEPO may stimulate ET generation [27, 28], while studies by Lebel et al and Hand et al showed no increase in plasma ET levels in HD patients treated with rhEPO [22, 29]. However, our data were not suggestive of increased but of decreased ET generation, implying that our finding of a reduced ET mediated tone is not affected by rhEPO. Furthermore, no correlation between the dose of rhEPO applied and the response to the ET-receptor antagonists was observed in our study.

In conclusion, we hypothesize that a reduced generation of ET in chronic renal failure provides the best explanation of our observations. Whether this is an epiphenomenon caused by "endothelial damage" related to uremia or whether uremic toxins simply suppress ET generation is unclear. As patients with diabetes, hypercholesterolemia, arterial hypertension or a history of smoking were excluded, which are common factors in these patients, our study is most likely representative of a smaller proportion of hemodialysis patients. We also emphasize that our studies were limited to the acute hemodynamic effects of ET antagonists. Chronic effects might be different from this short-term effect. Amann et al have shown in a uremic rat model that chronic ET-A receptor blockade prevented uremic vascular and cardiac remodeling [30]. It is conceivable that this effect of the ET system is at least as important as acute hemodynamic effects. Therefore, chronic studies in HD patients evaluating vascular and cardiac remodeling should be considered for implementation.

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